# Heterogeneric mating between two Podismini grasshopper species (Orthoptera, Acrididae, Melanoplinae)

Benjamín Jarčuška[1], Matúš Búci[1,2], Anton Krištín[1]

**1** Institute of Forest Ecology of the Slovak Academy of Sciences, Zvolen, Slovakia.
**2** Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, Zvolen, Slovakia.

Corresponding author: Benjamín Jarčuška (benjamin.jarcuska@gmail.com)

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## Abstract

The observation of mating between species of different genera in field conditions is extremely rare. Here, we report an instance of heterospecific copulation between a male of the Nagy's mountain grasshopper (*Pseudopodisma nagyi*) and a female of the green mountain grasshopper (*Miramella alpina*), which was observed in subalpine grasslands in the Veľká Fatra Mountains in central Slovakia. After being transferred to the laboratory, the female laid egg pods. This finding supports the hypothesis that the two species are closely related and suggests the need for a taxonomic revision within the tribe Podismini. If it occurs more often, reproductive interference may impact the viability of populations of both the green mountain grasshopper and Nagy's mountain grasshopper populations wherever they are in contact. Further data are needed to test whether reproductive interference reduces the viability of their syntopic populations.

## Keywords

Heterospecific copulation, interspecific mating, *Miramella*, *Pseudopodisma*, reproductive interference

## Introduction

Reproductive interference refers to sexual behavior between individuals of different species during the process of mate acquisition that results in a loss of fitness for one or both species (Gröning and Hochkirch 2008, Shuker and Burdfield-Steel 2017). Based on the temporal course of the reproductive behavior, different types of reproductive interference can be distinguished, ranging from disruption of sexual communication signals to late-acting processes such as successful copulation and hybridization. The boundaries between these types are gradual, and more than one type has been documented in many species pairs. Heterospecific copulations occur when premating barriers between the species are absent or inadequate or when one sex (usually the female) is unable to reject forced copulations from a heterospecific partner (Gröning and Hochkirch 2008). If such copulation has taken place, postmating pre- or postzygotic barriers hinder the process of hybrid speciation. Postmating barriers of hybridization in interspecific mating are thus associated with the highest fitness costs due to gamete wastage and energy and nutrient expenditure (Gröning and Hochkirch 2008, Shuker and Burdfield-Steel 2017).

Successful hybridization is confirmed by the appearance of hybrid individuals in the population, but most of these copulation attempts do not result in the production of viable offspring. Hybridization under field conditions between closely related Orthoptera species has been documented several times, albeit indirectly—primarily through molecular genetic analyses revealing gene flow between, for example, the Californian species *Melanoplus sanguinipes* (Fabricius, 1798) and *M. devastator* Scudder, 1878 (Orr et al. 1994) and between the European species *Chorthippus montanus* (Charpentier, 1825) and *C. parallelus* (Zetterstedt, 1821) (Rohde et al. 2015). Direct observations of heterospecific mating under field conditions are therefore anecdotal, with only one case reported for Orthoptera species (Gröning and Hochkirch 2008). The documentation of mating between representatives of different higher taxonomic categories (e.g., genus) is therefore even less likely (Sabaj and Peabody 1998, Mercado 2023). In Orthoptera, sexual interaction between species usually occurs through acoustic

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interference (Gröning and Hochkirch 2008). In two species that do not stridulate, heterospecific mating attempts or erroneous female choice are therefore more likely.

The green mountain grasshopper, *Miramella alpina* (Kollar, 1833) and the Nagy's mountain grasshopper, *Pseudopodisma nagyi* Galvagni & Fontana, 1995, belong to different taxonomic subtribes: Miramellina and Podismina, respectively (Cigliano et al. 2023). Nevertheless, their close phylogenetic relationship within the tribe Podismini (Orthoptera: Acrididae: Melanoplinae) suggests a shared evolutionary origin. European Podismini taxa are estimated to have diverged from their Asian counterparts approximately 38 million years ago (Litzenberger and Chapco 2001). Both species are less common in the Western Carpathians in Slovakia, and they are rare in Poland, Czechia, and Hungary. Both are mute (cannot stridulate due to very short forewings) and live in the mountains, preferably in grassland habitats between 600 and 1550 m.a.s.l. (Nagy et al. 2010, Chobanov et al. 2016, Hochkirch et al. 2016, Żurawlew et al. 2018–2023, Krištín 2025a, b). The species occur locally syntopically but only in Slovakia (Krištín et al. 2020). In this short note, we report a heterospecific copulation between a male of *P. nagyi* and a female of *M. alpina* observed in the field.

## Material and methods

The copulating pair was transported to the laboratory where it was kept in an insectary at 24 °C, 16/8 h light/dark cycle.

The embryonic developmental stage of the collected eggs was investigated on the basis of the external and internal egg structures after a single specimen of the preserved clutch had been dissected under a stereo zoom microscope (Kern OZP558, Kern and Sohn, Germany). The lipid yolk pieces in the dissected egg showed considerable differences in size, as is usually observed when a fertilized egg enters the early stages of embryogenesis (Bentley et al. 1979). To confirm genetic variation between the two species, we collected two males and two females from each species within the same population. DNA was extracted from the hind femur muscle tissue of the adult specimens and from the whole eggs using a high-salt protocol (Paxton et al. 1996). Partial sequences of the internal transcribed spacer 1 (ITS1) region were amplified to confirm the identity of the parental specimens. PCR amplification was performed using the following primers: 18S-28S [5′ TAG AGG AAG TAA AAG TCG 3′] (Weekers et al. 2001) and ITS-R1 [5′ CAT TGA CCC ACG AGC C 3′] (Ullrich et al. 2010). Each 15 µl PCR contained 7.50 µl PCRBIO Taq Mix Red (PCR Biosystems), 0.75 µl of each primer, 5 µl of ddH20, and template DNA. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 60 s, 52 °C for 120 s, and 72 °C for 120 s, with a final extension at 72 °C for 10 min. PCR products were purified using EPPiC Fast (A&A Biotechnology) and sequenced using the Sanger method. In total, four *P. nagyi* and five *M. alpina* individuals were successfully genotyped, including the female *M. alpina* from the copulating pair. The extracted genetic material from the parental specimens and/or specimens are deposited at the Institute of Forest Ecology of the Slovak Academy of Sciences in Zvolen, Slovakia.

## Results and discussion

During an orthopterological survey conducted in the Veľká Fatra Mts. (central Slovakia) on 27 July 2023, a mating pair consisting of female *M. alpina* and male *P. nagyi* was observed in copula (Fig. 1A). The observation site, Ostredok (48°53'51.480"N, 19°5'3.222"E), was a subalpine grassland on carbonates at an altitude of 1510 m.a.s.l. with a southeastern aspect (Fig. 1B). Both species were the most abundant among the 20 Orthoptera taxa recorded there (up to 30 adults per 100 $m^2$ for both species). No other copulating heterospecific pair was found at the site during the three-hour survey, while conspecific copulations were regularly observed in both species. No individuals with an obvious hybrid phenotype were observed at the site. After transporting the copulating pair to the laboratory, they copulated at least twice during the 54 days of life of both individuals. During this time, the female laid two oothecae. Genetic analysis of the ITS1 region confirmed the species identity of the parental specimens. Unfortunately, it was not possible to obtain a sufficient amount of DNA from the eggs for sequencing. The DNA sequences were deposited in the NCBI database under accession numbers PQ654838–PQ654846, with PQ654838 corresponding to the female *M. alpina* from the copulating pair.

Analyses of nuclear and mitochondrial DNA fragments reject the monophyly of Eurasian Podismini subtribes, which was previously proposed based on either morphological or cytological features (Chintauan-Marquier et al. 2014). Our observation is consistent with the evidence that the green mountain grasshopper and Nagy's mountain grasshopper are more closely related to each other than to other species within their subtribes (Litzenberger and Chapco 2001). The evolutionary proximity of parental species facilitates hybridization, as such relatives are more likely to be genetically compatible and can therefore produce viable offspring (Mallet 2005).

The syntopic occurrence of the studied species in the Western Carpathians is relatively rare, although they favor the same type of mountain grassland habitats. Sites where both species co-occur (n = 31) represent 14% of all recorded *M. alpina* sites and 35% of *P. nagyi* sites (n = 225 and 88 sites, respectively) in five Western Carpathian mountain ranges in Slovakia—Veľká Fatra, Nízke Tatry, Vysoké Tatry, Poľana, and Veporské vrchy (Krištín 2025a, b). This pattern can be explained by reproductive interference, which can reduce population viability wherever the two species are in contact (Gröning and Hochkirch 2008, Dorková et al. 2020). To gain deeper insight into the evolutionary consequences of heterospecific hybridization between these taxa, it is necessary to collect a range of hybrid specimens of different stages of development. Nevertheless, our observation provides further evidence that their current taxonomic status should be revised (Chintauan-Marquier et al. 2014, Cigliano et al. 2023). In particular, the shape of the genitalia, which is used to distinguish *Pseudopodisma* from *Miramella* (Kočárek et al. 2005), does not appear to prevent copulation between the two genera. The question remains as to whether copulation is also possible in the reverse position between these species.

**Fig. 1. A.** Copulation between a male of *Pseudopodisma nagyi* (top) and a female of *Miramella alpina* (bottom) in the Veľká Fatra Mountains (central Slovakia) on 27 July 2023. Both individuals were hosts of velvet mites (*Trombidium* sp.); **B.** The subalpine grassland habitat where the observation took place. Images by Benjamín Jarčuška.

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